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Phylogeographic analysis of barley DNA as evidence for the spread of Neolithic agriculture through Europe

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ABSTRACT

Results of analyses of the photoperiod response gene (PPD-H1) and simple sequence repeats (SSRs) in modern landraces of cultivated barley were used as evidence for the mechanism of agricultural spread in Neolithic Europe. In particular, we explored the usefulness of considering adaptive genes as indicators of past selective pressures acting on crops, during their spread through Europe. In some areas, such as the Alpine region, Britain and Scandinavia, we have evidence to suggest that the adaptation of crops to certain climatic conditions may have contributed to the timing of agricultural spread. At the northern fringes of Europe, and in higher altitude locations in central Europe, the introduction of more suitably adapted cereals may have facilitated successful agriculture to trigger agricultural expansion. This research opens up the possibility of investigating other genetic adaptations to climate, which would permit a fuller evaluation of the relative contributions of climate/crop and forager/farmer interactions in the process of agricultural spread.

1. Introduction

Evolutionary studies involving the genetic analysis of recently grown crop plants have tended to focus on the origins and initial domestication of cultivated plants from their wild progenitors (e.g. Heun *et al.* 1997; Badr *et al.* 2000; Özkan *et al.* 2002, 2010; Kilian *et al.* 2007; Luo *et al.* 2007; Morrell and Clegg, 2007; Allaby *et al.* 2008; Jones *et al.* 2008b; Brown *et al.* 2009; Allaby, 2010). The usefulness of similar methods for addressing questions relating to the subsequent spread of agriculture out of its region of origin might be questioned because archaeological and historical sources provide evidence for extensive movement of crops through trade, exchange or population movements since their initial introduction into Europe. The link between recent and Neolithic cereal populations therefore requires careful consideration before phylogeographic analysis is used to provide information on the early spread of agriculture. This paper presents evidence to show that traditional local landraces of barley apparently retain, at least partially, a genetic record of their prehistoric origins, and then uses this source of evidence to investigate early agricultural spread in Europe.

It is generally accepted that cereal cultivation was introduced into SE Europe in c.7000 BC and, over the next c.3000 years, spread through Europe by two principal routes, one following the Danube and Rhine river valleys through central Europe and from there into the North European Plain, and the second taking a route along the Mediterranean coast through Italy to Iberia (e.g. Bogucki, 1996; Price, 2000; Davison *et al.* 2006; Tresset and Vigne, 2011). Agriculture spread rapidly through the Balkans (the area of the Starcevo-Cris-Körös Neolithic cultures) but appears to have slowed down in the Great Hungarian Plain (Halstead, 1989; Zvelebil and Lillie, 2000; Kertész and Sümegi, 2001; Whittle *et al.* 2002; Bocquet-Appel et al., 2009) before again spreading rapidly through central Europe (Linearbandkeramic – LBK – culture). There were apparently further delays in the establishment of agriculture in the North European Plain (e.g. Funnel Beaker – TRB – and Corded Ware cultures; Zvelebil and Rowley-Conwy, 1984; Bogucki, 1996; Zvelebil and Lillie, 2000; Rowley-Conwy, 2011), and the Alpine region (e.g.

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Cortaillod and Pfyn cultures; Bogucki, 1996; Gkiasta *et al.* 2003). The spread of agriculture through the Mediterranean appears to have been relatively rapid.

Various theories have been advanced to explain the apparent delays in the spread of agriculture, including the slow assimilation of agricultural practices by existing indigenous human populations, due to the relative success of the hunter-gatherer lifestyle in environments rich in wild resources (e.g. Zvelebil and Rowley-Conwy, 1984, 1986; Bogucki, 1996; Zvelebil and Lillie, 2000), and theories based on the time taken for crops to adapt to novel, or changing, climatic conditions (e.g. Halstead, 1989; Bogucki, 1996, 2000; Bonsall et al., 2002; Gronenborn, 2007; Gyulai, 2007). Crop genetics, by providing direct evidence for the adaptation of crops to climatic conditions, can potentially shed light on the relative contribution of climatic and cultural factors in determining the rate of agricultural spread in different regions. In this paper, therefore, we present DNA evidence for crop adaptation and spread, based on recently grown plants.

2. Genetic typing of European barley landraces

A landrace has been defined as "a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems" (Camacho Villa et al., 2005). Many landraces have died out locally over the last century, but seeds collected from all parts of Europe are available from germplasm collections (Jones *et al.* 2008a). We have investigated the utility of this source of evidence through the analysis of European barley landraces and two types of genetic marker: simple sequence repeats (SSRs or 'microsatellites'), neutral genetic loci that contain repeat DNA sequences in units usually between one and six nucleotides in length, and the adaptive photoperiod response gene (PPD-H1), responsible for the control of flowering time in relation to day length, and which had previously been shown to display genetic diversity in cultivated barley (Laurie *et al.* 1995; Turner *et al.* 2005).

A total of 651 accessions of barley landraces from across Europe were selected from a larger collection of 2560 accessions, in order to provide, as far as possible, full geographical coverage without bias towards any particular phenotypes. The 651 accessions were analysed for 24 different SSRs, each of which displays a variable number of repeat sequences, depending on the particular accession (Jones *et al.* 2011). The version (allele) possessed by an individual accession was typed by the polymerase chain reaction (PCR), the length of the PCR product indicating the number of repeats present. For 148 of these landraces, the PPD-H1 locus was also sequenced (Jones *et al.* 2008b). A single nucleotide polymorphism (SNP) is responsible for the adaptive response of the PPD-H1 gene, one allele (*Ppd-H1*) causing the plant to respond to lengthening days by coming into flower, and the other allele (*ppd-H1*) resulting in a plant that is not responsive to lengthening days. In addition to the landraces for which the PPD-H1 locus was sequenced, the causative SNP in PPD-H1 was identified (as either responsive or non-responsive) for a further 82 landraces (Jones *et al.* 2011). Both the SSRs and the PPD-H1 locus were subjected to statistical analysis to identify populations or haplotype groups, which were used to explore relationships among the landraces. These analyses included the construction of networks and phylogenetic trees that enable the evolutionary relationships between landraces to be investigated, and the use of a Bayesian clustering method to place landraces into groups of genetically-defined populations (Brown and Brown, 2011: 35–37).

Network analysis of the PPD-H1 haplotypes revealed population structure in the cultivated barley landraces (Jones *et al.* 2008b). Three distinct groups of European haplotypes were seen within the networks. All day-length non-responsive haplotypes cluster together in a single group (A). The other two groups (B and C) contain only day-length responsive haplotypes. The barley SSR dataset also displayed population structure (Fig. 1), with the most likely number of distinct populations lying between eight and eleven (Jones *et al.* 2011). The consistent assignment of SSR populations at different levels in a hierarchical structure, and the deep-rooted association of landraces from the same population in the phylogenetic tree constructed from the SSR data (Fig. 1), indicate that the populations are authentic and not merely an artefact of the method of analysis. For the purposes of phylogeographic interpretation, a population structure of eleven SSR populations has been chosen, which includes two pairs of populations (1A&B; 6A&B) that are genetically more closely related to one another than to any of the other populations.

3. The validity of landraces as a source of genetic information on the early spread of barley

For modern landraces to be a useful source of information on questions concerning early agricultural spread, it is clearly necessary that they have some antiquity, and have not become redistributed and mixed to such an extent that their 20th century locations reveal nothing about their past phylogeography. Phylogeographic patterns of landraces may be created through bottleneck effects, which reduce the genetic variation of a crop population, when it spreads into an area in which it was not previously found. This could involve the spread of a crop species into an area where it was not previously cultivated, or the spread of a new strain (genotype) of a particular crop into an area where a different strain of the same species already exists. Subsequent movements within the same area will tend to obscure this original pattern, by mixing previously distinct populations, the members of which may homogenise through cross-pollination, rather than create new patterns (cf. Lister *et al.* 2009). So, if existing European landraces were primarily the result of crop movements that have occurred within Europe since the introduction of agriculture, little of the original geographic pattern would be expected to survive. The only patterns discernable would be those created and maintained through new selective pressures arising since this genetic homogenisation. So the existence of geographic patterns in modern landraces would suggest that they are a relic of spread into new areas with no, or different, pre-existing cultivars. To investigate this, both SSR populations and PPD-H1 haplotype groups were mapped geographically, to identify phylogeographic patterning that might relate to the early spread of agriculture through Europe, and compared in terms of their known phenotypic characteristics (Jones *et al.* 2008b, 2011). Several aspects of these mapping exercises support the view that modern European landraces do retain some phylogeographic information relating to the initial spread of agriculture.

Both the PPD-H1 haplotype groups and the SSR populations display geographic patterning when landraces are plotted on a map of Europe, and some of this geographic structure is apparently not determined by phenotype. The phenotypic characteristics recorded for each landrace included physical characteristics such as ear row number (determined by the number of fertile spikelets per rachis node), which distinguishes two-row barley (with one fertile spikelet per node) from six-row barley (with three fertile spikelets per node), caryopsis structure, i.e. whether the grain was hulled (with lemma and palea fused to the surface of the grain) or naked (with free lemma and palea), as well as physiological characteristics such as growth habit (winter or spring), and flowering in response to day length (Jones *et al.* 2011). The physical characteristics were

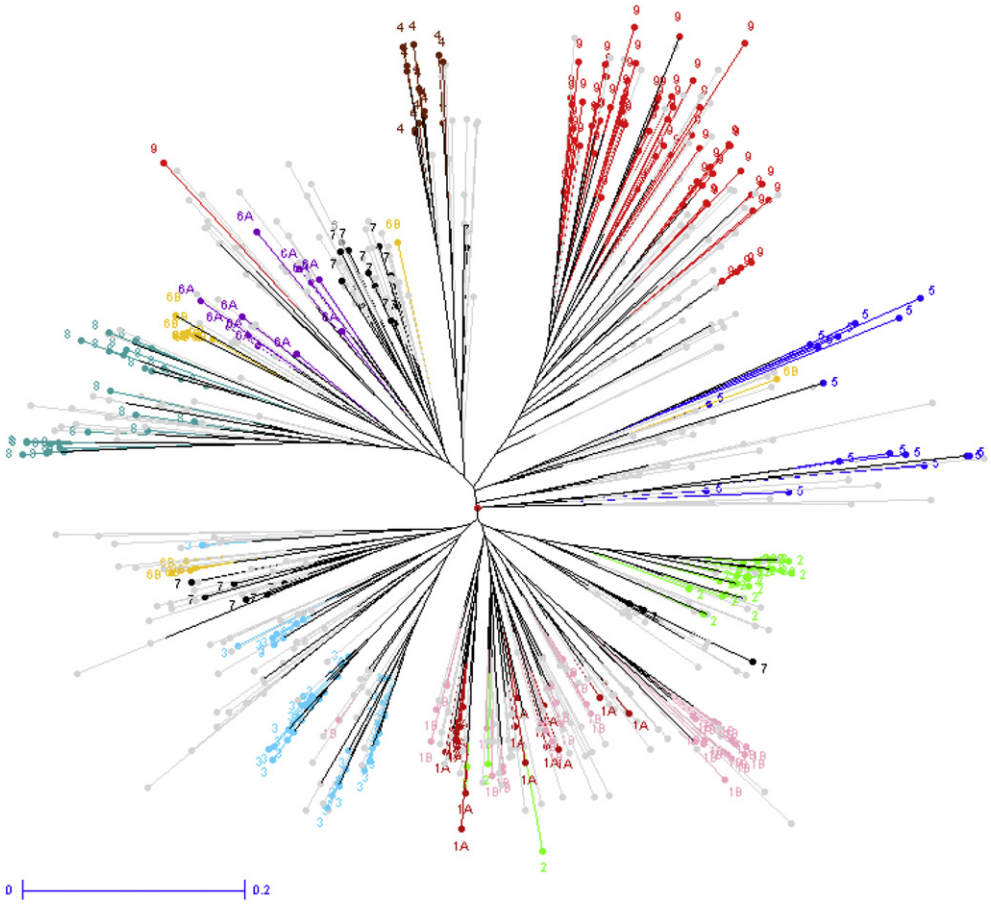

Fig. 1. Phylogenetic (neighbour-joining) tree constructed from SSR data. Numbers and colours indicate different SSR populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

taken from information supplied with the accessions and from personal observation of grain morphology. Growth habit information was supplied with the accessions and also, for a subset of landraces, predicted from genes responsible for the requirement of vernalisation (exposure to a cold period for the initiation of flowering; Cockram *et al.* 2007); photoperiod response was inferred, for a subset of landraces, from the photoperiod response gene (see above; Jones *et al.* 2008b, 2011).

Geographic structure unrelated to phenotype is indicated first by the geographic distribution of the two European groups of responsive PPD-H1 haplotypes (B and C, Fig. 2). As well as possessing the same allele for photoperiod response, these two groups of landraces have shown no other indication of phenotypic difference in growth studies (Jones *et al.* 2008b), and they show no strong differences in other recorded phenotypic characteristics (Table 1). Their distribution is therefore unlikely to be the result of current selective pressure. Group B landraces predominate in southern Europe in regions with a Mediterranean coast, and Group C landraces are largely distributed across Europe from the northern Balkans to western Europe and the North European Plain. These geographic distributions show a strong similarity to those expected if Group B had spread into Europe mainly by a southern route along the Mediterranean, and Group C by a northern route through central Europe, (and then further north into Britain and Scandinavia), following the well-documented trajectories of Neolithic agricultural spread. This suggests that modern landraces preserve at least some of the phylogeographic pattern set up during the initial Neolithic spread of agriculture.

Secondly, most of the SSR populations are geographically restricted to some extent. Two of the SSR populations (9 and 6A) show a strongly Mediterranean distribution, while seven of the other populations (1A&B, 2–4, 6B, 7) are centred in central and northern Europe (Fig. 3). Of this second group, population 7 predominates in Scandinavia, populations 1A and 2 are concentrated in NW Europe (including Britain), and 1B and 4 in central Europe. Most landraces in populations 3 and 6B are tightly clustered in the Alpine region of Switzerland and the Great Hungarian Plain respectively. Populations 5 and 8 are widely distributed. There is clear evidence for overlapping distributions of these populations (Fig. 3), which may well result from relatively recent crop movements, but the fact that geographically distinct genetic groups can be identified suggests that some of the phylogeographic patterning relating to the introduction of barley into Europe may remain. The contrast between the wide distribution of the Mediterranean population 9 and the relatively restricted distributions of most of the populations in central and northern Europe may be a consequence of the rapid spread of agriculture along the Mediterranean coast compared to the punctuated spread further north, and therefore also an indication that these populations reflect the early spread of agriculture.

SSRs are neutral genetic markers that do not contribute directly to phenotype, and so are not themselves subject to selective pressure. However, if a group of landraces is maintained as a separate population because those landraces possess an important phenotype, then over time they will acquire a distinct genetic make up, such as an SSR signature. Several of the SSR populations retain

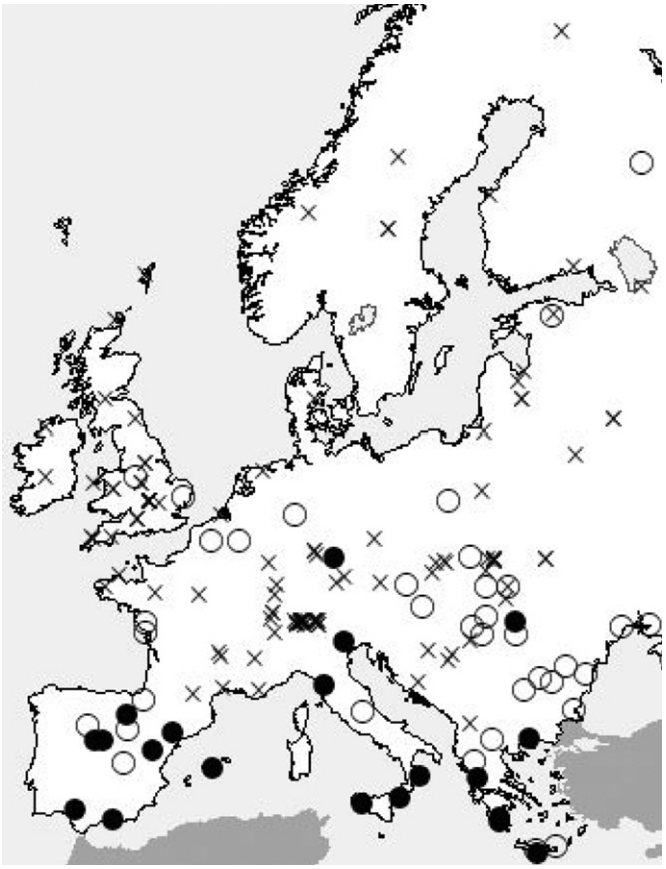

Fig. 2. Geographic distribution of PPD-H1 haplotype groups of European barley landraces. Crosses = Group A, Filled symbols = Group B, Open symbols = Group C.

ancestral wild-type phenotypes, such as winter growth habit, day-length responsiveness and hulled grains, suggesting that they may be relicts of the early spread of barley into Europe, and most display equally deep origins in the neighbour-joining phylogenetic tree, indicating that they are of comparable antiquity (Jones *et al.* 2011). No single recorded phenotype is associated with just one population, suggesting that none of the populations have arisen as a result of recent phenotypic selection. Indeed most of the recorded phenotypes are widely distributed (Fig. 4). Smaller scale phylogeographic investigations of SSR populations in Spain and Italy also suggest that modern landraces retain at least some of the phylogeographic pattern set up during the initial spread of agriculture into Europe (Yahiaoui *et al.* 2008; Isaac *et al.* 2010).

4. Rates, routes and mechanism of agricultural spread through Europe

There is some evidence of phenotypic differentiation between SSR populations (Table 2; Jones *et al.* 2011). The most distinctive phenotypic group is made up of the central/north-western European populations 1–3, where over 90% of landraces were 2-row, hulled, spring barleys which, of those typed for PPD-H1, were predominantly (98%) non-responsive to day length. The majority of the predominantly spring landraces from populations 4 (100%) and 7 (92%), in north-east Europe and relatively high altitude locations in central Europe, were also non-responsive to day length (100% and 68% respectively of spring landraces typed for PPD-H1). This is consistent with predictions by Turner *et al.* (2005) that delayed flowering (non-responsiveness to day length) is advantageous (particularly for late germinating spring barleys) in northern latitudes, where the growing season is long due to moist summers, as it permits an extended period of vegetative growth into the summer season. Day-length non-responsiveness may also be important at higher altitudes, where the onset of the growing season is likely to be later, and so an extended growth period particularly advantageous, especially if growth rate is slower due to cooler conditions. The geographic distribution of these SSR populations is therefore likely to be maintained by this environmental pressure, and it has also been suggested, on the basis of lesser genetic diversity, that populations 1–3 represent a secondary introduction of barley into Europe (Jones *et al.* 2008b, 2011).

On the other hand, the landraces of the Mediterranean population 9 (which are also mostly spring barleys) are predominantly responsive to day length (Table 2; 88% of the spring/facultative barleys are responsive to day length). This is again consistent with predictions by Turner *et al.* (2005) that the promotion of early flowering through response to lengthening days is an advantage in an area with dry summers and a short growing season, as it enables plants to avoid the summer drought. Population 6A, also from the Mediterranean, is phenotypically similar (largely six-row, hulled, with a predominantly spring habit – Table 2), but these two populations have a different evolutionary history, with PPD-H1 group B landraces predominating in population 9, and groups A and C in population 6A.

Population 6A is mainly located in Greece, Italy and the southern Balkans, and may be a relic of the original introduction of barley into Greece and its initial spread north into the Balkans and west along the Mediterranean. It is genetically very similar to population 6B, most of which is tightly clustered in the Great Hungarian Plain. Population 9 may represent the rapid spread of a different strain of barley throughout the Mediterranean. Populations 6 and 7 are affiliated, in both the neighbour-joining phylogenetic tree and the hierarchical population structure, suggesting that they were originally part of a single population (Jones *et al.* 2011) that may have evolved during the spread of barley through central and northern Europe. There is some evidence to suggest that population 7 (and to a lesser extent population 6), acquired the day-length non-responsive characteristic through hybridisation with populations 1–3 (Jones *et al.* 2011), when the latter arrived in Europe, and the same may be true for population 4, which is grouped with populations 6 and 7 in the phylogenetic tree.

There is very little spread of population 6B beyond the Hungarian Plain into central Europe, which could therefore represent the recent 'end-product' of a bottleneck that restricted the passage of this population type further west and north. Further north, population 7 is the only population with a significant number of landraces in Scandinavia, in this case potentially representing a population that has emerged at the other end of a bottleneck in the North European Plain. A particularly tight SSR cluster of landraces (population 3) is located in the Alpine region of Switzerland. It is highly unlikely that the recent locations of these populations correspond exactly to the localities in which they originally arose, given that they are, by definition, usually associated with traditional farming systems, and so tend to be found in restricted locations. Nevertheless, all three of these general areas

Table 1
Phenotypic characteristics for PPD-H1 European barley haplotype groups.

PPD-H1 haplotype group	Growth habit			Row number			Grain type		
	% Spring	% Winter	*N*	% 2-Row	% 6-Row	*N*	% Hulled	% Naked	*N*
A	95	5	147	78	22	148	94	6	149
B	53	47	17	5	95	21	100	0	21
C	57	43	44	14	86	44	100	0	44

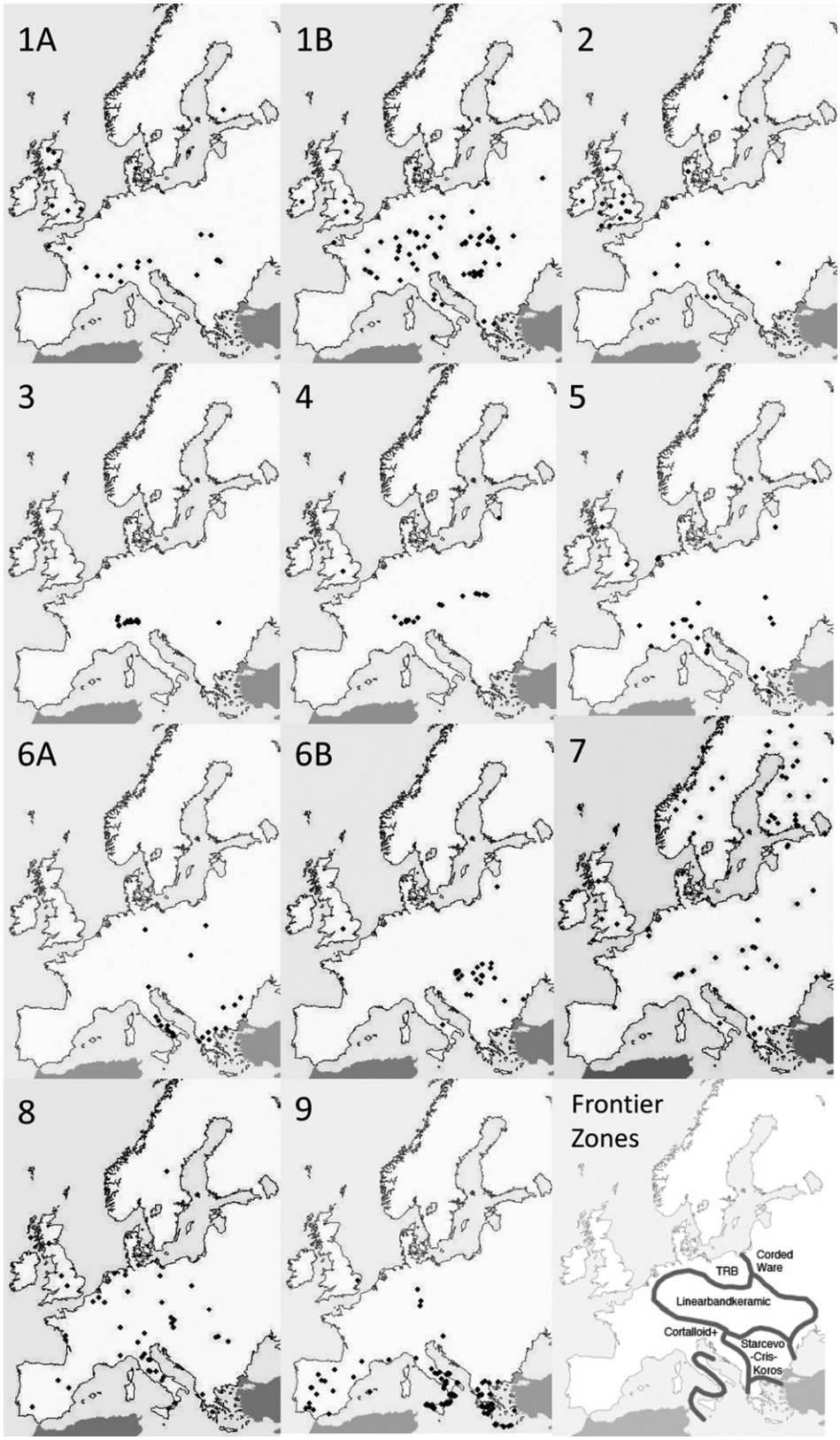

Fig. 3. Geographic distribution of SSR populations of European barley landraces. Numbers indicate SSR populations. Neolithic agricultural frontier zones redrawn after Zvelebil and Lillie 2000. Pottery cultures: 1 = Greece, 2 = Starcevo-Cris-Körös, 3 = Linearbandkeramic (LBK), 4 = Funnel Beaker (TRB), 5 = Corded Ware, 6 = Cortaillod, Pfyn and other cultures of the Alpine region, 7 = Italian Impressed Ware, 8 = West Mediterranean Impressed Ware.

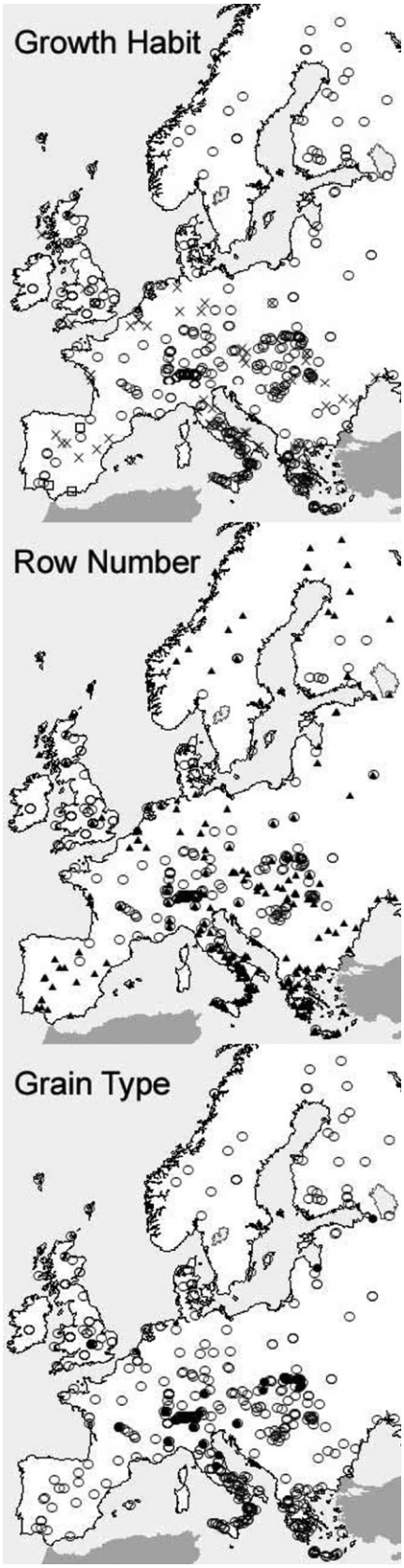

Fig. 4. Geographic distribution of phenotypic characteristics of European barley landraces. Growth Habit: Crosses = winter barley, Circles = spring barley, Squares = facultative barley (barley with a spring genotype but sown in autumn in regions of mild winter climate). Row Number: Circles = two-row barley, Triangles = six-row barley. Grain Type: Open circles = hulled barley, Filled circles = naked barley.

have been identified as points along the northern trajectory of spread where there may have been a slowing down of agricultural expansion (e.g. Kertész and Sümegi, 2001; Gkiasta *et al.* 2003; Halstead, 1989; Bogucki, 1996; Zvelebil and Lillie, 2000; Bocquet-Appel *et al.* 2009).

Indeed, the primary locations of these SSR populations seem to respect some of the major frontier zones defined as areas of sustained interaction between foraging and farming communities as agriculture spread through Europe during the Neolithic (Fig. 3; Zvelebil and Dolukhanov 1991, Zvelebil and Lillie, 2000). For example, the northern boundary of Hungarian population 6B landraces corresponds to the frontier zone identified between the Starcevo-Cris-Körös cultures of the Balkans and the subsequent Linearbandkeramik (LBK) culture of central Europe (Zvelebil and Lillie, 2000). Other frontier zones are indicated to the south of the LBK region separating it from the later Cortaillod, Pfyn, and other cultures of the Alpine region (where population 3 is primarily located), and north of the LBK area separating it from the later cultures of the north European Plain (Funnel Beaker (TRB) and Corded Ware cultures), beyond which population 7 predominates. Another frontier zone runs NW to SE through Italy, at the westerly extent of population 6A between the Impressed Ware cultures of southern Italy and the west Mediterranean.

If the geographically concentrated SSR populations do represent the end result of a series of genetic bottlenecks, these may be due to simple restriction of the gene pool contributing to the founder population in the new area or they may be due to past selection of a particular adaptive trait (Doebley *et al.* 2006). The latter could potentially shed light on the mechanism of agricultural spread by identifying the selective pressure (environmental or human) acting on the adaptive trait. For example, acquisition of day-length non-responsiveness by a group of early barley cultivars that had already evolved a spring growth habit could have been a key evolutionary adaptation that enabled cereal cultivation to be extended further west and north into the regions now occupied by population 7. In modern cereal populations, however, it is difficult to distinguish genetic traits selected for in the distant past from those maintained only through current selective pressure. The day-length non-responsive *ppd-H1* phenotype does not appear to be an essential adaptation for the cultivation of barley in central Europe, however, as responsive *Ppd-H1* types are quite common in this area (Fig. 2). A lack of this adaptation is therefore unlikely to be the cause of a genetic bottleneck in Hungary accounting for a delay in the spread of agriculture at that point. This does not rule out an environmental barrier as an explanation for a delay in the spread of agriculture into central Europe, as other adaptive genes may have been key to the spread of crops into this area, but we have found no convincing evidence that adaptation to increased summer precipitation acted as a barrier to the spread of barley at this frontier.

There are relatively few occurrences of day-length responsive types in Britain and Scandinavia, and day-length non-responsiveness would undoubtedly have been advantageous for the predominantly spring barleys in the Scandinavian part of population 7 (Jones *et al.* 2011) and for the spring barleys of populations 1A, 1B and 2 which make up the bulk of landraces from Britain. In southern Scandinavia, barley (along with emmer wheat) was one of the principal crops in the 4th millennium BC, and increased relative to emmer at the beginning of the 3rd millennium (Robinson, 2003, 2007). Barley and emmer were also the major crop species from c. 4000 cal BC onwards in Neolithic Britain (Jones and Rowley-Conwy, 2007; Bishop *et al.* 2009). It is possible, therefore, that the introduction of a day-length non-responsive type of barley helped to facilitate the relatively late establishment of successful agriculture in these northerly regions of Europe. The wetter climate has been postulated as a main cause for the expansion of barley cultivation

Table 2
Phenotypic characteristics and PPD-H1 haplotype groups for European barley SSR populations.

SSR Population	Row number			Caryopsis type			Growth habit			Daylength responsiveness			PPD-H1 haplotype groups			
	% 2-Row	% 6-Row	*N*	% Hulled	% Naked	*N*	% Spring	% winter	N	% non-resp	% responsive	N	%A	%B	%C	N
1A	98	2	43	100	0	44	100	0	42	100	0	4	100	0	0	4
1B	99	1	86	95	5	87	99	1	83	100	0	27	100	0	0	27
2	98	2	60	100	0	60	93	7	60	95	5	37	100	0	0	35
3	94	6	77	97	3	77	100	0	76	100	0	30	100	0	0	30
4	71	29	28	25	75	28	100	0	28	100	0	12	100	0	0	12
5	36	64	36	72	28	36	69	31	32	75	25	9	75	0	25	8
6A	4	96	27	100	0	27	65	35	26	42	58	12	42	17	42	12
6B	19	81	27	100	0	27	67	33	27	25	75	12	25	8	67	12
7	22	78	86	100	0	91	92	8	91	61	39	38	61	0	39	38
8	14	86	57	100	0	57	35	65	51	0	100	23	0	11	89	9
9	6	94	109	99	1	109	68	32	105	8	92	25	8	68	24	25

into central Sweden and Scotland (Kirleis et al., 2012), which fits well with the notion that a day-length non-responsive type of barley was available at this time.

Bonsall *et al.* (2002) have suggested, however, that a period of drier (and warmer) conditions at around 4000 cal BC facilitated the spread of agriculture into Britain and Scandinavia at this time by extending the growing season in spring. A climatic change of this type could interact with photoperiod response in different ways. On the one hand, an earlier start to the growing season could reduce the need for an extension of the growing season later into the summer through day-length non-responsiveness; on the other hand, a combination of early growth onset (due to climatic 'improvement') and an extended growth period later in the year (due to day-length non-responsiveness) could be particularly advantageous, and non-responsive barleys might have been among the first crop types to thrive in this climatic window of opportunity. Bonsall *et al.* (2002) have also suggested that similar climatic changes may be relevant to upland areas of Europe such as the Alps and Carpathians, and it is interesting that a significant proportion of the more southerly members of population 7 are arranged along this mountain chain (Fig. 3). Only a few of these have been typed for PPD-H1, however, and these are mostly responsive to day length.

On the other hand, nearly half of the tightly clustered group of landraces in Switzerland (population 3, Fig. 3) were sequenced for PPD-H1, and all were non-responsive. Although cultivated crops were present at LBK sites in the northern Alpine Foreland from the second half of the 6th millennium cal BC, the quantities of cereal remains increase substantially in the 4th millennium cal BC, including at Alpine lakeshore settlements (Jacomet, 2007), perhaps indicating an increased role for cultivation at this time. Barley, in particular, is found in much greater quantities at these later sites, and it is tempting to suggest that a new strain of day-length non-responsive barley was introduced at this time, accounting for its increased importance. So, in this case too, it may well have been, at least partly, environmental constraints that delayed the establishment of fully agricultural communities until better adapted cereal strains (such as day-length non-responsive barleys) became available. The same adaptation may also have facilitated the later bronze and iron age spread of crops into higher altitude locations of the eastern Alps, where again barley was the predominant cereal (Schmidl *et al.* 2005, 2007).

During the Neolithic, the barley in central Europe, the Alpine region and southern Scandinavia was, however, predominantly naked (Bakels, 1991, 2007; Kirleis et al., 2012; Jacomet, 2007; Robinson, 2003, 2007) and probably six-row, whereas the recent non-responsive barley of SSR populations 1–3 is predominantly hulled and two-row. This may point to a later introduction of the non-responsive type of barley. On the other hand, another group of barleys (SSR population 4), which is concentrated in the Alpine and Carpathian region, is also day-length non-responsive for all those landraces sequenced for PPD-H1, but predominantly (75%) naked, and more mixed in terms of ear type, while the non-responsive barleys which predominate in SSR population 7, the only SSR population that is common in Scandinavia, are nearly all six-row. It is equally possible, therefore, that the hulled and two-row characteristics of populations 1–3 were acquired through later introgressions from other populations.

5. Conclusions

For farming communities, the impact of climatic variation is likely to be felt most acutely through its effects on crops, either directly as a food source or indirectly through their use as fodder. The adaptive response of crops therefore provides a particularly good source of information on the role of climate in the spread of farming. The analysis presented here has argued that it is possible, up to a point, to distinguish recent landrace populations that reflect the initial introduction of cultivated crops during the early spread of Neolithic agriculture through Europe from those that result from mixing due to subsequent crop movements or from later introductions. It may therefore be possible to use the evidence of these landraces to consider the relative roles of climatic adaptation and cultural assimilation as factors explaining the mechanism of early agricultural spread. We have some evidence that the adaptation of crops to certain climatic conditions may have contributed to the delay and subsequent spread of agriculture in some areas, such as the Alpine region, where the introduction of more suitably adapted cereals may have helped to facilitate successful agriculture, and at the northern fringes of Europe, where these cereals may also have interacted with climatic fluctuations to trigger agricultural expansion. This is not to say that prolonged interaction between farmers and hunter-gatherers did not also play a significant role in the delay of agricultural spread at these frontiers, and at other frontiers for which we have found no evidence of crop adaptation.

As far as we are aware, this is the first attempt to use evidence of an adaptive gene as an indicator of past selective pressures acting on crops to provide evidence for the mechanism of agricultural spread, though adaptive genes have been successfully used to investigate the domestication process in maize (Jaenicke-Després *et al.* 2003; Wright *et al.* 2005). Further research into the evolution and phylogeography of a greater range of genetic adaptations to climate, such as cold tolerance, would permit a fuller evaluation of the relative contributions of climate/crop and forager/farmer interactions in the process of agricultural spread, as would the investigation of other crop species. This type of research would also be greatly assisted by a method for reliably distinguishing between past and present selection of relevant traits, in order to date the selective pressures acting on crops during their evolutionary

history, and so identify the natural and human factors providing the mechanism of spread and delay, both chronologically and geographically. Ancient DNA could provide this link, if it can be extracted from a sufficient number of early charred cereal assemblages (see Bunning *et al.* 2012; for a recent development in this area), and would have the great advantage of being directly relatable to palaeoclimate. In the absence of ancient DNA, methods for the analysis of modern landraces that could give at least a relative order of trait selection would provide useful evidence for the selective pressures, both human and environmental, acting on crops at different points in time. Exploiting the time depth of extant specimens, in particular pre-20th century specimens, also holds great potential (cf. Lister *et al.* 2010). These approaches, in turn, could yield information, not only on the mechanism of agricultural spread but also on human requirements and priorities relating to the cultivation and use of crop species, and how these changed through time.

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